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(54) Title: STIMULATION OF HAIR GROWTH WITH POTASSIUM CHANNEL OPENERS AND 5 $\alpha$ -REDUCTASE INHIBITORS			
(57) Abstract			
An improved method and composition for promoting hair growth in mammals for treating various forms of alopecia or male pattern baldness comprising the concomitant administration of potassium channel openers, such as minoxidil, cromakalim, pinacidil, or a compound selected from the classes of s-triazine, thiane-1-oxide, benzopyran and pyridinopyran compounds; and a 5 $\alpha$ -reductase inhibitors, 17 $\beta$ -(N- <i>tert</i> -butylcarbamoyl)-4-aza-5 $\alpha$ -androst-1-en-3-one. The method comprises administering an effective amount of the potassium channel opener and administering an effective amount of the 17 $\beta$ -(N- <i>tert</i> -butylcarbamoyl)-4-aza-5 $\alpha$ -androst-1-en-3-one whereby the promotion of hair growth is increased over the sole administration of the potassium channel opener.			

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**Stimulation of Hair Growth with Potassium Channel Openers  
and 5 $\alpha$ -Reductase Inhibitors**

**BACKGROUND OF THE INVENTION**

5        The present invention relates to improved methods and compositions for promoting hair growth by the concomitant administration of a potassium channel opener such as minoxidil, cromakalim, pinacidil, or a compound selected from the classes of potassium channel openers such as s-triazine derivatives, benzopyran derivatives, pyridinopyran derivatives and thiane-1-oxide compounds; and a 5 $\alpha$ -reductase inhibitors, 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5 $\alpha$ -androst-1-en-3-one.

10      Since the discovery that minoxidil could promote hair growth and was useful in the treatment of androgenetic alopecia commonly known as "male pattern baldness" (US Patents 4,596,812; 4,139,619), alopecia areata, and balding in females, effort has been directed toward attempts to improve upon the sole use of a topical minoxidil composition by incorporating other active 15 ingredients, for example the combinations of minoxidil/hydrocortisone and minoxidil/retinoids (DE 3827467-A).

20      Another approach has been to attempt to identify and quantify the biological mechanisms responsible for the initiating and controlling hair growth at the follicular level and intervening with therapeutic agents which stimulate hair growth. It is well known in the art that the onset of puberty results in changes in endocrine levels which result in, among other changes, the stimulation of 25 facial hair growth (males), axillary hair growth (males and females), and pubic hair growth (males and females). One class of compounds implicated in these effects are the androgens.

25      Androgens appear to play a major role in the development and progression of androgenetic alopecia (male pattern baldness). Rittmaster, R. S., *Clin. Dermatol.*, 6, 122 (1988). This was demonstrated in humans, in that prepubertally castrated men do not develop male pattern baldness. 30 Hamilton, J. B., *Am. J. Anat.*, 71, 451 (1942). In the skin, and presumably in the follicle, the effector androgen is dihydrotestosterone (DHT), the product of the enzymatic conversion of testosterone to DHT by the enzyme 5 $\alpha$ -reductase. Ebling, F. J. *Clin. Endocrinol. Metab.*, 15, 319 (1986); Price, V. H. *Arch. Dermatol.*, 111, 1496 (1975). Additional evidence for DHT's role 35 stems from reports that men with male pattern baldness have elevated 5 $\alpha$ -reductase levels in the hair follicles and skin of the frontal scalp and that men with a 5 $\alpha$ -reductase deficiency do not develop male pattern baldness. Bingham, K. D., Shaw, D. A., *J. Endocrinol.*, 57, 111 (1973); Schweikert, H. U., Wilson, J. D., *J. Clin. Endocrinol. Metab.*, 385, 811 (1974).

35      Antiandrogen therapy offers a potential intervention to the treatment of male pattern baldness in men, and in women where it has been suggested that elevated androgen levels are responsible for the appearance of male pattern baldness. Rittmaster, R. S., Loriaux, D. L., *Ann. Intern. Med.*,

106, 95 (1987). This class of compounds, the antiandrogens, have been subdivided into two major categories, the androgen receptor blockers and the  $5\alpha$ -reductase inhibitors. Androgen receptor blockers interfere with the binding of androgens, testosterone and dihydrotestosterone (DHT), to their receptors, while  $5\alpha$ -reductase inhibitors prevent the conversion of testosterone into DHT.

5 The clinical use of androgen blockers, such as cyproterone acetate, in men is complicated with systemic effects on normal sexual function. This is not the case in women, where these agents are utilized. Burke, B. M., Cunliff, W.J., *Br. J. Dermatol.*, 112, 124 (1985); Dawber, R. P. R., Sonnex, T., Ralfs, I., *Br. J. Dermatol.*, *Suppl.* 107, 20 (1982). The other class of antiandrogens are the  $5\alpha$ -reductase inhibitors. These compounds block action of  $5\alpha$ -reductase and thus reduce 10 the level of DHT in the peripheral tissue. Rittmaster, R. S., Stoner, E., Thompson, D. L., Nance, D., Lasseter, K. C., *J. Androl.*, 10, 259 (1989). Brooks, J. R., Berman, C., Primka, R. L., Reynolds, G. F., Rasmusson, G. H., *Steroids*, 47, 1 (1986). Rittmaster, R. S., Uno, H., Povar, M.L., *J. Clin. Endocrinol. Metab.*, 65, 188 (1987) have shown that topical treatment of periadolescent stumptail macaque monkeys with the  $5\alpha$ -reductase inhibitor 4-MA prevented the 15 progression of baldness normally observed in this species. Similar studies in humans have not been reported.

The coupling of hair growth stimulation with potassium channel activation has been inferred from literature reports of several vasodilators, minoxidil (through its metabolite minoxidil sulfate), pinacidil, and diazoxide, which cause varying degrees of hypertrichosis upon oral administration.

20 Zins, G.R., *Clin. Dermatol.*, 6, 132 (1988); Goldberg, M.R., *J. Cardiovasc. Pharmacol.*, 12(*Suppl. 2*), S41 (1988); Okun, R., Russell, R.P., Wiklson, W.R., *Arch Inter. Med.*, 112, 886 (1963). It is also known in the art that topical minoxidil is an effective treatment for androgenetic alopecia. The mechanism by which these compounds, minoxidil (through its metabolite minoxidil sulfate), pinacidil, and diazoxide, dilate vascular smooth muscle has been reported to be via the 25 opening (activation) of potassium channels. Robertson, D. W., Steinberg, M. I., *J. Med. Chem.*, 33, 1529 (1990). Thus potassium channel openers are useful in the treatment of androgenetic alopecia.

The surprising and unexpected result, and the subject of this invention, is the synergistic effect observed when both  $17\beta$ -(N-tert-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one and minoxidil, a 30 potassium channel opener, are utilized to stimulate or promote hair growth in a statistically significant fashion when compared to each drug alone.

#### INFORMATION DISCLOSURE STATEMENT

Topical minoxidil has been shown to be an effective treatment for male pattern baldness as described in US Patents 4,596,812 and 4,139,619. The utilization of  $17\beta$ -(N-tert-butylcarbamoyl)-35 4-aza-5- $\alpha$ -androst-1-en-3-one for the treatment of androgenetic alopecia has been disclosed in

-3-

European Patent Application 285 382 A2. The use of combinations of androgen receptor blocking agent (antiandrogen) and 5 $\alpha$ -reductase inhibitors (not 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one) has been disclosed in US Patent 4,684,635. The combination of minoxidil and an androgen receptor blocking agent for the treatment of male pattern baldness has also been disclosed in Patent Applications DE 3615-396-A and WO 8700-427-A. The combination of minoxidil and a 5 $\alpha$ -reductase inhibitor for the treatment of male pattern baldness has also been disclosed in Japanese Patent Application JA 1305-017-A.

#### SUMMARY OF THE INVENTION

In one aspect, the subject invention is directed toward an improved method for promoting hair growth in mammals comprising the administration of a potassium channel opener in an effective amount and the administration of 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one in an effective amount whereby hair growth is increased over the sole administration of the potassium channel opener. The routes of administration for either component can be by any of various means, preferably orally, topically or any combination of the two. More preferably and conveniently, the two components are applied together topically.

The potassium channel opener can be administered topically in an amount of from about .01 to about 20 percent by weight, or orally in an amount of from about .001 to about 50 mg/kg body weight. The 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one can be administered topically in an amount of from about 0.01 to about 10 percent by weight of composition or administered orally in an amount of from about 0.001 to about 10 mg/kg body weight. The potassium channel opener and/or 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one is generally administered in a pharmaceutical carrier adapted for oral administration or in a pharmaceutical acceptable carrier adapted for topical application.

Preferably, the potassium channel opener and 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one are combined into a pharmaceutical carrier adapted for topical application. The potassium channel opener can be routinely applied to an area of treatment concomitant with the administration of 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one.

The potassium channel opener is minoxidil, cromakalim, pinacidil, or a compound selected from the chemical classes of s-triazines, thiane-1-oxides, benzopyrans, pyridinopyrans and derivatives thereof or a pharmaceutically acceptable salt thereof.

In another aspect, the subject invention is directed toward a pharmaceutical composition comprising an effective amount of a potassium channel opener, an effective amount of 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one and a pharmaceutical carrier adapted for topical application. The pharmaceutical carrier can be petrolatum, lanolin, propylene glycol, N-methyl-2-pyrrolidinone, polyethylene glycol, oleyl alcohol, ethyl alcohol or mixtures thereof.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for promoting hair growth which means to increase normal hair growth or restore hair growth in mammals, including humans, suffering from hair growth disorders such as alopecia or male pattern baldness. The promotion or restoration of hair growth has been discovered to be significantly enhanced by the concomitant administration of a 5 potassium channel openers (activators); and a 5 $\alpha$ -reductase inhibitor, 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one.

Typical examples of "potassium channel openers" or "potassium channel opener vasodilators" as contemplated by the subject invention are minoxidil, cromakalim, pinacidil, and those 10 compounds selected from the chemical classes of s-triazines, benzopyran, pyridinopyran and thiane-1-oxides their derivatives and pharmaceutically acceptable salts.

Minoxidil is chemically, 6-amino-1,2-dihydro-hydroxy-2-imino-4-piperidinopyrimidine and analogs thereof. The preparation of these compounds are described in U.S. Patents 3,382,247, 3,461,461 and 3,644,364 and J.M. McCall, et al., *J. Org. Chem.*, 40, 3304 (1975). Related 15 compounds are sulfoxypyrimidinium, -pyridinium, and -triazinium which are described in U.S. Patent 4,287,338 herein incorporated by reference. Hereinafter, the term "minoxidil" means any of the various forms of 6-amino-1,2-dihydro-hydroxy-2-imino-4-piperidinopyrimidine, derivatives and analogs thereof. Minoxidil is distributed by The Upjohn Company, Kalamazoo, MI.

Cromakalim is chemically, (3S-trans) 3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-2H-1-benzopyran-6-carbonitrile, a molecular wight of 286.33 g. and a melting point of 224.5-225.5°C. Cromakalim is distributed by SmithKline Consumer Products, Philadelphia, PA.

Pinacidil is chemically, N-cyano-N'-4-pyridinyl-N''-(1,2,2-trimethylpropyl)-guanidine monohydrate, a molecular weight of 263.34 g. and a melting point of 110-116°C. The preparation 25 of pinacidil is described in US Patent 4,057,636 and German Patent 2,557,438 and is distributed by Eli Lilly and Company, Indianapolis, IN.

s-Triazine compounds or 2,6-diamino-4-substituted-s-triazine-1-oxides are described in U.S. Patent 3,270,014 assigned to The Upjohn Company, Kalamazoo, MI. Specific examples of these compounds include: N4-hexyl-2,4,6-triamino-1,3,5-triazine-1-oxide; N4-butyl-2,4,6-triamino-1,3,5-triazine-1-oxide; N4-pentyl-2,4,6-triamino-1,3,5-triazine-1-oxide; 4-(N,N-dipropyl)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(N,N-dibutyl)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(1-pyrollidinyl)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(N,N-di(2-methyl-2-propenyl)amino)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(N,N-(diallylamino)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(N,N-dimethylamino)-2,6-diamino-1,3,5-triazine-1-oxide; 4-(1-piperidinyl)-2,6-diamino-1,3,5-triazine-1-oxide; N4-propyl-2,4,6-triamino-1,3,5-triazine-1-oxide; N4-ethyl-2,4,6-triamino-1,3,5-triazine-1-oxide; N4-(1,1,4,4-tetramethylbutyl)-2,4,6-triamino-1,3,5-triazine-1-oxide; and N4-heptyl-2,4,6-triamino-1,3,5-

triazine-1-oxide.

Thiane-1-oxide compounds are described in U.S. Patent 4,568,682 assigned to Rhone-Poulenc Sante, Courbevoie, France. An example of such a compound contemplated by the subject invention is N-methyl-2-(pyridin-3-yl)tetrahydrothiopyran-2-carbothioamide-1-oxide.

5 Other potassium channel openers include pyranopyridine derivatives described in patent applications GB 2 204 868 A and benzopyran derivatives described in patent applications GB 2 204 868 A, EP 314 446 A2, EP 339 562 A, EP 340 718 A, EP 337 179, AU A 18556/88, JA 1294 677 A, EP 359 537 A, and US Patent 4,900,752.

10 Pharmaceutically acceptable salts of the potassium channel openers are for example acid addition salts which may be chosen from the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 15 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate.

15 Pharmaceutically acceptable cationic addition salts of the potassium channel openers include: pharmacologically acceptable metal cations, ammonium, amine cations, or quaternary ammonium cations. Especially preferred metal cations are those derived from the alkali metals, e.g., lithium, 20 sodium, and potassium; and from the alkaline earth metals, magnesium and calcium, although cationic forms of other metals, e.g., aluminum, zinc, and iron are also within the scope of this invention. Pharmacologically acceptable amine cations are those derived from primary, secondary, and tertiary amines.

25 The second essential, active ingredient of the subject invention is the compound 17 $\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one, (hereinafter referred to as "TBCAA") which has a molecular weight of 372.56. This compound is a 5 $\alpha$ -reductase inhibitor and is described in European Patent Application 88302807.8, Publication No. 0285382, published 5 October 1988, assigned to Merck & Co., Inc., Rahway, NJ. This publication discloses various analogs of TBCAA all of which are disclosed to be active as testosterone 5 $\alpha$ -reductase inhibitors and thus are 30 useful for the treatment of androgenic alopecia, including male pattern alopecia. The preferred administration of TBCAA is topical. TBCAA can be administered by any of various routes as well as can be the potassium channel opener.

35 Administration routes for the two components of the subject invention can be topically, orally, parenterally or rectally. Typically, TBCAA is administered either topically with the potassium channel opener or orally while the potassium channel opener is applied topically. Preferably and most conveniently, the administration route would be to compound TBCAA with a potassium

channel opener in a pharmaceutical, topical vehicle.

Typically, the potassium channel opener and optionally the  $17\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one compound are applied to the skin region where hair growth is desired with a pharmaceutical carrier. More preferably, the pharmaceutical carrier is adopted for topical application such as those pharmaceutical forms which can be applied externally by direct contact with the surface to be treated.

Conventional pharmaceutical forms for this purpose include ointments, waxes, gels, lotions, pastes, jellies, sprays, aerosols, and the like in aqueous or nonaqueous formulations. The term "ointment" embraces formulations (including creams) having oleaginous, absorption, water-soluble 10 and emulsion-type bases, e.g., petrolatum, lanolin, polyethylene glycols, N-methyl-2-pyrrolidinone, oleyl alcohol as well as mixtures of these.

Preparation of minoxidil topical compositions are disclosed in U.S. Patents 4,139,619 and 4,596,812, both herein incorporated by reference, as examples of how to prepare topical compositions for any of the potassium channel openers and/or the  $17\beta$ -(N-*tert*-butylcarbamoyl)-4-15 aza-5- $\alpha$ -androst-1-en-3-one.

Additionally, the potassium channel openers and/or the  $17\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one compound can be admixed with other compounds for the treatment of hair growth. Such compounds which can be included in the overall composition or treatment are various combinations of the potassium channel openers, vasoconstrictors such as betamethasone 20 dipropionate, corticosteroids such as hydrocortisone, scopolamine, and antiandrogens such as cyaotol, and cyproterone acetate.

Typically, a potassium channel opener is used in an effective amount that is an amount sufficient to promote hair growth or treat hair growth disorders such that hair growth is increased or produced. The potassium channel opener is added in an amount of from about 0.001 to about 25 10, preferably, 0.01 to about 5 percent by weight of the composition.

The  $17\beta$ -(N-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one compound is used in an effective amount, that is, an amount sufficient to promote hair growth or treat hair growth disorders such that hair growth is increased or produced over that which would be increased or produced by the administration of a potassium channel opener alone. The testosterone 5 $\alpha$ -reductase inhibitor, 30 TBCAA, is added in an amount of from about 0.0001 to about 10, preferably, 0.001 to about 5 percent by weight of the composition.

In a topical application, the compound or formulated composition can be applied to the area to be treated, in mammals such as the scalp in humans, by spraying, dabbing or swabbing. Other less specific methods can be employed provided the active ingredient(s) are delivered to the region 35 of a hair follicle. Preferably, the compound or formulated composition is periodically applied to the treatment area on a routine basis prior to, during and subsequent to hair growth. Generally,

the routine treatment would be to apply the compound or formulated composition at least daily, preferably twice daily although more frequent applications can be used.

The percentage by weight of the active ingredients, potassium channel opener and  $17\beta$ -(*N*-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one compound herein utilized ranges from an effective amount which is an amount sufficient to increase normal hair growth or treat various forms of alopecia whereby the hair growth is significantly more than if either of the compounds were solely administered. In topical preparations the pharmaceutical carrier for topical applications constitutes a major amount of the preparation. Typically, the active ingredient is in a range of from about 0.01 to about 10 percent total weight of the topical composition, preferably 0.1 to 5 percent total weight.

Experimentation:

The following protocol was utilized with the stumptail macaque monkey to demonstrate the synergistic affect of minoxidil and  $17\beta$ -(*N*-*tert*-butylcarbamoyl)-4-aza-5- $\alpha$ -androst-1-en-3-one ("TBCAA") for promoting hair growth.

Twenty-one male stumptail macaque (*Macaca speciosa*) monkeys were assigned to vehicle control and drug treated groups on the basis of baseline hair weight data. This assignment procedure was necessary to insure that the average baseline hair growth for each control and experimental group was comparable. The control and drug treatment groups were as follows:

1. Topical 50:30:20 vehicle (N=6)
- 20 2. Oral TBCAA and topical 50:30:20 vehicle (N=5)
3. Oral TBCAA and topical 100mM minoxidil (N=5)
4. Topical 100mM minoxidil (N=5).

The vehicle consisted of 50% propylene glycol, 30% ethanol, and 20% water. The 100mM concentration of topical minoxidil was formulated in this vehicle. TBCAA was prepared as an oral dose of 0.5mg per monkey (interanimal weight range of 9.5-15.5 kg). Immediately prior to the dosing phase of the study, hair was removed from a 1 inch square area (identified by four tattoos) in the center of the balding scalp. This hair collection was the baseline hair growth determination prior to the beginning of treatment. Approximately 250 $\mu$ L of vehicle or 100mM minoxidil (prepared in vehicle) were topically administered to the tattooed area of the scalp. For the groups which received combined topical and oral dosing, 0.5 mg/monkey TBCAA was ingested by the monkeys at the same time as the topical dose was administered. The monkeys were dosed once per day, seven days per week for twenty weeks.

At four week intervals throughout the dosing phase of the study, each monkey was shaved and the hair was collected and weighed. The body weight data (at baseline and during assay) were analyzed by the nonparametric Wilcoxon rank-sum test. Differences were significant at  $p < 0.05$ . The hair weight data (mean  $\pm$  SEM) at each 4 week collection for vehicle and treatment groups

were expressed as the change from baseline. Statistical analysis (ANOVA) was performed on the ranks of the data to show overall differences among groups at each 4 week collection with  $p < 0.10$  marginally significant,  $p < 0.05$  significant, and  $p < 0.01$  highly significant.

Results:

5 The data reported in Tables I and II were obtained after 12 weeks of dosing, Tables III and IV after 16 weeks of dosing and in Tables V and VI after 20 weeks of dosing. They show that the combination of TBCAA and minoxidil is statistically superior in promoting hair growth, in this model, than the constituent agents alone.

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